IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Frank LUYTEN et al.	Confirmation No.:	5817
Serial No.:	10/089,994	Art Unit:	1632
Filed:	July 2, 2002	Examiner:	Thaian N. Ton
Customer No.:	21559		
Title:	ISOLATION OF PRECURSOR CELLS AND THEIR USE FOR TISSUE REPAIR		

DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. FRANK LUYTEN

1. I am a named inventor on the above-referenced patent application.

2. I am a Professor at the University of Leuven. I have over 20 years of experience in the field of Rheumatology. A copy of my curriculum vitae is attached.

3. I have read and understand the Final Office Action mailed December 15, 2006. In particular, I understand that the Examiner has questioned whether the evaluation of cartilage production in nude mice is a reliable model for a therapeutic effect. The Examiner has indicated that the working examples show in vivo implantation of the cells by intramuscular injection of the cells into nude mice, which, the Examiner has indicated is not considered analogous to what could be considered a therapeutic treatment. The Examiner has indicated that the examples fail "to correlate to a therapeutic result in utilizing the claimed cells" (*see*, Final Office Action, page 8, second paragraph).

4. In the invention described in the application under consideration, it is demonstrated that, based on the correlation with cartilage production upon injection into nude mice, markers can be identified, which are representative of the ability of the cells to produce stable hyaline cartilage when injected *in vivo*. It was found that the ability of cells to produce stable hyaline cartilage when injected *in*

vivo, is linked to the expression of specific markers by these cells prior to injection. Moreover it was found that precursor cells of chondrocytes are similarly capable of producing stable hyaline cartilage when injected in vivo, and that accordingly, representative markers of this cartilage-forming ability can be identified.

5. That a cell population expressing the markers for cartilage-forming ability are indeed also capable of producing stable hyaline cartilage *in vivo*, when injected into a cartilage defect is further supported by the enclosed data (Annex). These data demonstrate that the expression, by a cell population obtained from a biopsy, of markers which have been identified to be representative of cartilage forming ability in vivo using the nude mouse model, is indicative of the ability of the cell population of producing stable hyaline cartilage when injected into a cartilage defect, and thus representative of the therapeutic potential of that cell population. Accordingly, these data confirm that the markers identified by the *in vivo* nude mouse model, are reliable tools to identify whether or not a cell population obtained from a biopsy is suitable for use in the therapy of cartilage defects using autologous cell transplantation. The application demonstrates that, using the same *in vivo* nude mouse model, the relevant markers for chondrocyte precursors cells were identified. As demonstrated in the enclosed data, the markers allow predictable determination of whether or not these cells can improve cartilage formation in cartilage defects. Accordingly it is submitted we have thus provided methods and tools for identifying therapeutically useful precursor cell populations.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: _____ _____

Dr. Frank Luyten

ANNEX

Experimental data

1) Identification of markers for the ability to produce stable hyaline cartilage in vivo using the nude mouse model

Markers for the ability of cells to produce stable hyaline cartilage *in vivo* were identified using the nude mice model as described in the specification. Briefly, cell populations were injected intramuscularly into nude mice and the ability of the cell populations to produce stable hyaline cartilage was monitored. The markers specifically expressed by those populations which, when injected, led to stable hyaline cartilage formation in mice, were identified as markers of chondrocyte phenotypic stability.

2) Use of the markers to identify populations for implantation into a cartilage defect

In a randomized, well-controlled, level I-1a evidence clinical trial, the biomarkers identified during the phase (1) were used to identify by molecular screening *in vitro* cartilage biopsies from patient suffering from osteoarthritis, populations of cells capable of producing hyaline cartilage *in vivo* for re-injection into the patients. Patients were first evaluated to obtain a Baseline score (BSL) of the defect. Healthy cartilage was then harvested from an unaffected part of the joint. The cell populations obtained from the were assessed for the expression of the relevant markers by molecular screening. The expression of these markers was attributed a score (C-C). The cells were then further cultured before being re-implanted into the cartilage defect in the patient.

Clinical improvement was evaluated at 12 to 18 months after implantation.

3) Correlation between the expression of the biomarkers and the clinical outcome

The score used to measure clinical improvement is the well-validated Knee Osteoarthritis Outcome Score (KOOS). Another measurement was done based on patient-reported questionnaire that measures pain, symptoms, activity of daily living, sports and recreational activities and quality of Life (QOL score).

A statistically significant positive correlation was observed between the expression of the biomarkers for chondrocyte phenotypic stability by the cell populations prior to injection, and the average clinical improvement as measured by KOOS and QOL scores (*see*, Figures 1 and 2, respectively).

4) Conclusion

Based on the above it is concluded that the expression by a cell population of the markers identified in the nude mouse model as indicative of chrondocyte phenotypic stability, is representative of the therapeutic potential of the cells when injected in a cartilage defect.

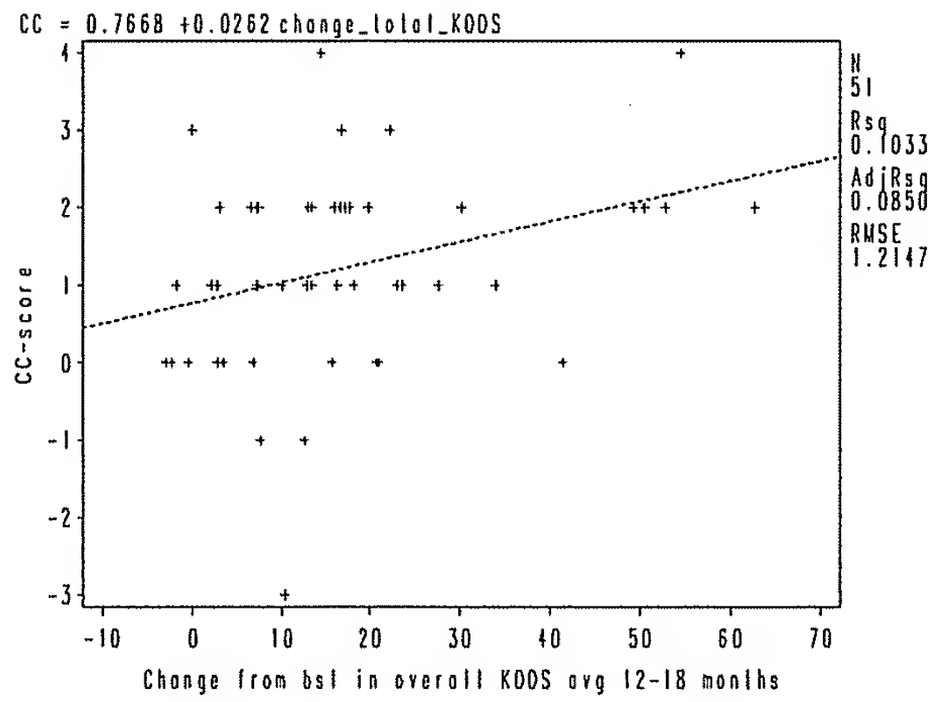

Figure 1: correlation between CC-score and change in overall KOOS (Pearson correlation coefficient: 0.32138; p=0.0215)

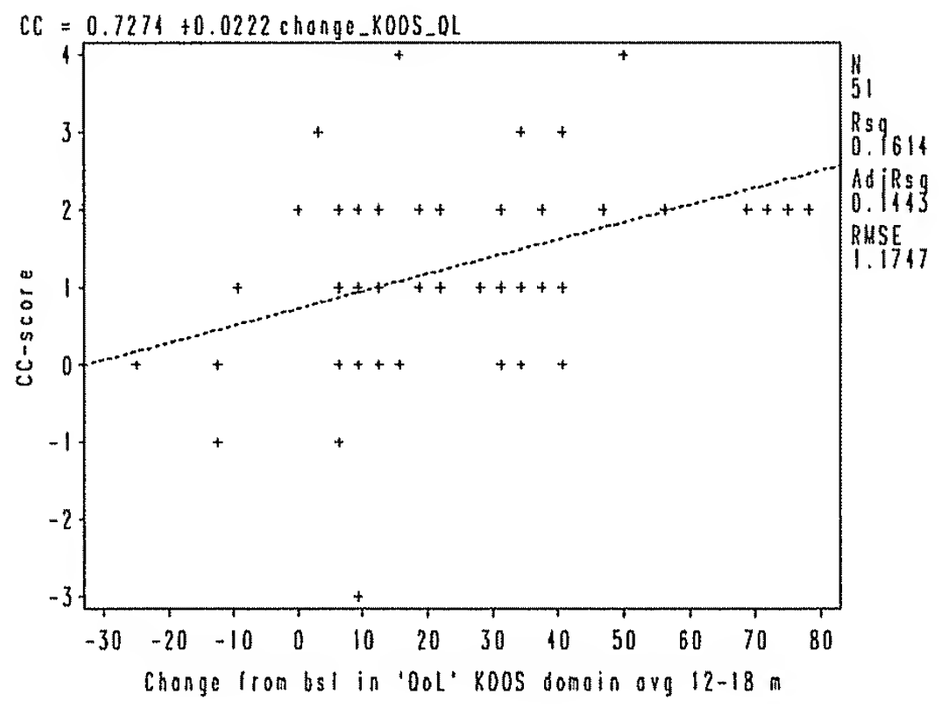

Figure 2: correlation between CC-score and change in the quality of life subscore of KOOS (Pearson correlation coefficient: 0.40172; p=0.0035)